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Note

Evaluation of BMPA, MWY, GVPC and BCYE media for the isolation of *Legionella* species from respiratory samples☆

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ABSTRACT

Culture media performance is a critical factor in the isolation of *Legionellae* from respiratory samples. We showed that BMPA and MWY media yielded significantly higher isolation rates than GVPC and BCYE media in regard to performance with samples that harbored low *Legionella* inocula and high contamination levels.

Legionnaires' disease (LD), a severe form of pneumonia, is mainly diagnosed by urinary antigen detection. Nevertheless, *Legionellae* isolation from respiratory secretions by culture is considered the gold standard for diagnosis because of its superior specificity. All *Legionella* species and serogroups can be detected by culture, as it allows epidemiological typing and susceptibility testing.

The reported culture sensitivities range widely from less than 10% to 80%, reflecting the difficulties in improving the isolation of this fastidious and slow-growing bacteria (Murdoch, 2003). Laboratories experienced in *Legionellae* isolation are more likely to recover the organism. Delays in respiratory sample processing, the prior use of antimicrobial therapies and culture overgrowth by other oropharyngeal bacteria are additional factors that limit the culture yields.

Over the last decades, specific formulated media have been developed, resulting in several buffered charcoal yeast extract (BCYE) agars with or without selective added agents that inhibit competing flora (Edelstein, 1981). The culture medium quality is a critical factor in *Legionellae* isolation. Several studies have been performed to compare these selective media during *Legionellae* isolation from water samples (Edelstein, 1982, Luck et al., 2004, Reinthaler et al., 1993, Ta et al., 1995). However, their performances in *Legionellae* isolation from human samples have not yet been evaluated. Therefore, we performed a comparative assessment of four commercially available media for *Legionellae* recovery from respiratory samples.

This prospective study included 328 patients with suspected LD who were admitted to French hospitals from September 2010 to June 2012. A LD case was defined as a patient with clinical and/or radiological findings compatible with pneumonia and at least one of the following positive tests for *Legionella*: urinary antigen, axenic culture, amoebic coculture, *Legionella*-specific PCR and nested sequence-based typing (Descours et al., 2012, Ginevra et al., 2009, Jarraud et al., 2013). A total of 328 respiratory samples (127 sputa, 61 tracheo-bronchial aspirations (TBA) and 140 broncho-alveolar lavages (BAL)) were collected. If necessary, the sputa were liquefied with dithiothreitol (Sputasol®, Oxoid, Dardilly, France).

The non-selective medium used in this study was the buffered charcoal yeast extract (BCYE) agar (Oxoid). The selective media were BCYE supplemented with cefamandole (4 mg/L), polymyxin B (80,000 UI/L)

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and anisomycin (80 mg/L; BMPA medium; Oxoid); BCYE supplemented with glycine (3 g/L), vancomycin (1 mg/L), polymyxin B (80,000 UI/L) and cycloheximide (80 mg/L; GVPC medium; BioMérieux, Marcy l'Etoile, France) and BCYE supplemented with glycine (3 g/L), vancomycin (1 mg/L), polymyxin B (50,000 UI/L) and anisomycin (80 mg/L; MWY medium; Oxoid). The axenic culture was performed within 24 h of arrival at the laboratory. Specimens that were not immediately plated were refrigerated at 4 °C until processed. From each sample, 100 μL were inoculated without heat or acid pretreatment onto five plates, 1 BCYE, 2 BMPA, 1 GVPC and 1 MWY. The inoculated plates were incubated for 10 days at 35 °C in an aerobic atmosphere (BCYE, BMPA) or in a 2.5% CO_2 atmosphere (BMPA, GVPC, MWY) and inspected on days 3, 5 and 10 after inoculation. The presumptive colonies were subcultured on BCYE agar plates and serogrouped with latex agglutination (Slidex® *Legionella* kit, BioMérieux) and polyclonal antibodies produced in our laboratory for indirect fluorescent antibody staining (Jarraud et al., 2013).

Among the 328 patients, 183 LD cases were diagnosed; these comprised 178 *Legionella pneumophila* and 5 other *Legionella* species. A total of 96 samples were culture-positive for *L. pneumophila* according to one to five plates (sensitivity, 52.5%), including 93 samples with *L. pneumophila* serogroup 1 (Lp1), and 1 each with Lp2, Lp5 and Lp8. No other *Legionella* species grew. Most cultures (95.7%) were positive on days 3 or 5. The sampling type did not lead to statistically significant differences in the culture performances ($p = 0.079$, Chi-Square test).

Significant differences in *Legionella* recovery from the respiratory samples were observed for the five inoculated plates ($p < 0.001$, Chi-Square test). Their respective performances were BMPA (2.5% CO_2), 41%; MWY (2.5% CO_2), 40.4%; BMPA (air), 37.7%; GVPC (2.5% CO_2), 25.7% and BCYE (air), 21.3%. As described by Feeley et al. on BCYE, we observed a slightly greater number of colonies on BMPA plates incubated in a 2.5% CO_2 atmosphere than in BMPA plates incubated on air, which did not reach a statistical significance ($p = 0.52$, Chi-Square test) (Feeley et al., 1979, Feeley et al., 1978). The differences observed between the five inoculated plates correlated with low *Legionella*-load samples (<10 CFU/plate) ($p < 0.0001$, Chi-Square test) (Fig. 1). Similarly, significant differences in sample contamination by oropharyngeal flora were observed ($p < 0.001$, Chi-Square test) (Fig. 2). BMPA and MWY were the most sensitive and least contaminated media. The combined isolation rate for the BMPA and MWY plates yielded a sensitivity of 48.1%.

When selecting an appropriate culture method, maximal sensitivity for the isolation of *Legionella* strains is desirable. A major factor is the quality of the culture media used. To our knowledge, there are no published data regarding the performances of commercially available agar plates, although *Legionella* isolation from respiratory samples is performed in several laboratories.

We found that selective media supplemented with antibiotics and antifungals yielded significantly higher isolation rates than BCYE medium, as demonstrated by Leoni and Legnani with hot water systems (Leoni and Legnani, 2001). Among these, the higher recovery rates

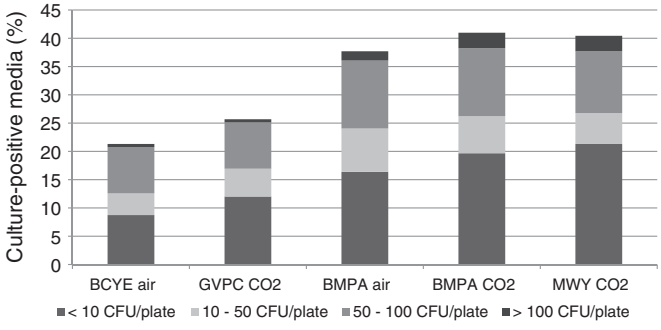

Fig. 1. Media performances with regard to the number of *Legionella* colonies on BCYE, GVPC, BMPA and MWY plates.

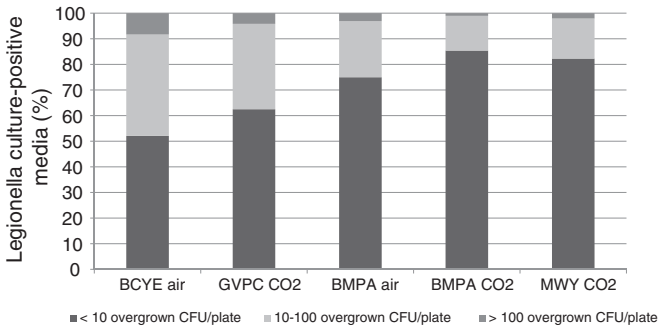

Fig. 2. Other bacterial overgrowth of BCYE, GVPC, BMPA and MWY among *Legionella* culture-positive media.

obtained with MWY and BMPA media were related to their performances with samples that harbored low levels of *Legionella* inocula and their low contamination levels. Their performances may be related to anisomycin B, an antifungal component inhibiting the growth of oropharyngeal yeasts and missing in GVPC medium. Several authors demonstrated similar results with hot water samples, whereas Reinthaler et al. observed no differences between culture media before heat or acid pretreatment (De Luca et al., 1999, Edelstein, 1982, Luck et al., 2004, Reinthaler et al. 1993). Our results suggest that the combination of MWY and BMPA plates provides an efficient *Legionella* isolation rate from respiratory specimens. The use of a combination of GVPC and BCYE is not recommended.

This work was limited to the comparison of four plates provided by two different manufacturers. A comparison of the performances of MWY, BMPA and GVPC media from several suppliers for the isolation of *Legionella* would complete this study, although Luck et al. have ever demonstrated no significant difference between four GVPC media for the isolation of *Legionella* from water samples. Moreover, the strains isolated in our study were all *L. pneumophila*. The glycine and cefamandole contained in these media have been shown to possibly inhibit the growth of any non-*pneumophila* species (Lee et al., 1993, Ta et al., 1995). On the basis of this literature data, the addition of BCYE to MWY and BMPA plates may be recommended when other *Legionella* species are suspected. Finally, sample processing by heat or acid buffer pretreatment, which was not evaluated in this study, is also critical to increase the culture sensitivity by reducing the overgrowth of other bacteria (Buesching et al., 1983, Edelstein et al., 1982). The combination of an optimal culture media choice and sample pretreatment could enhance the isolation of *Legionella* strains from overgrown respiratory samples.

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